Evolution of *Pseudomonas aeruginosa* chronically infecting cystic fibrosis patients

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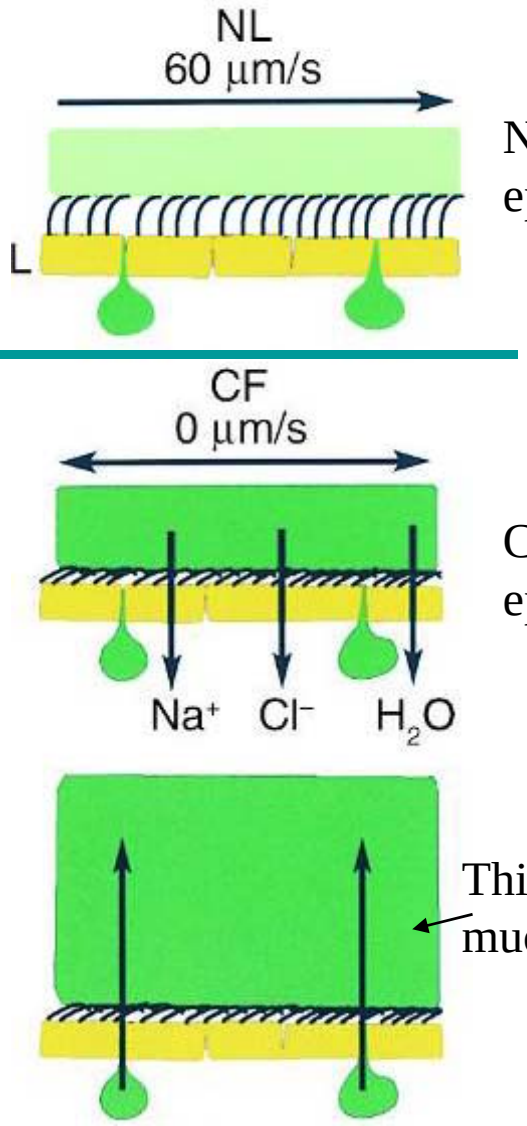
The Biosystem

- bacterial migration from the open environment to human airways

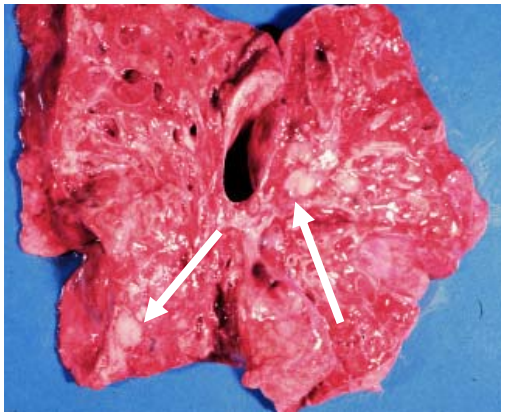
Pseudomonas aeruginosa infections in Cystic Fibrosis patients

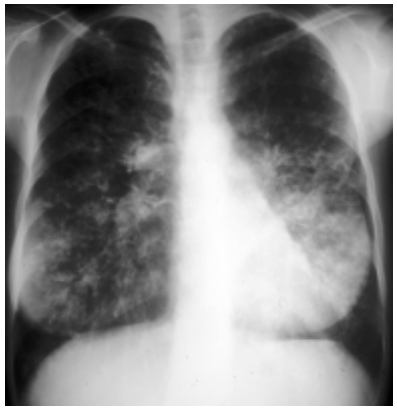
- CF is caused by mutations in the *CFTR* gene which affect chloride channels.

- This results in decreased fluidity of mucus → Impaired clearance of inhaled microbes.

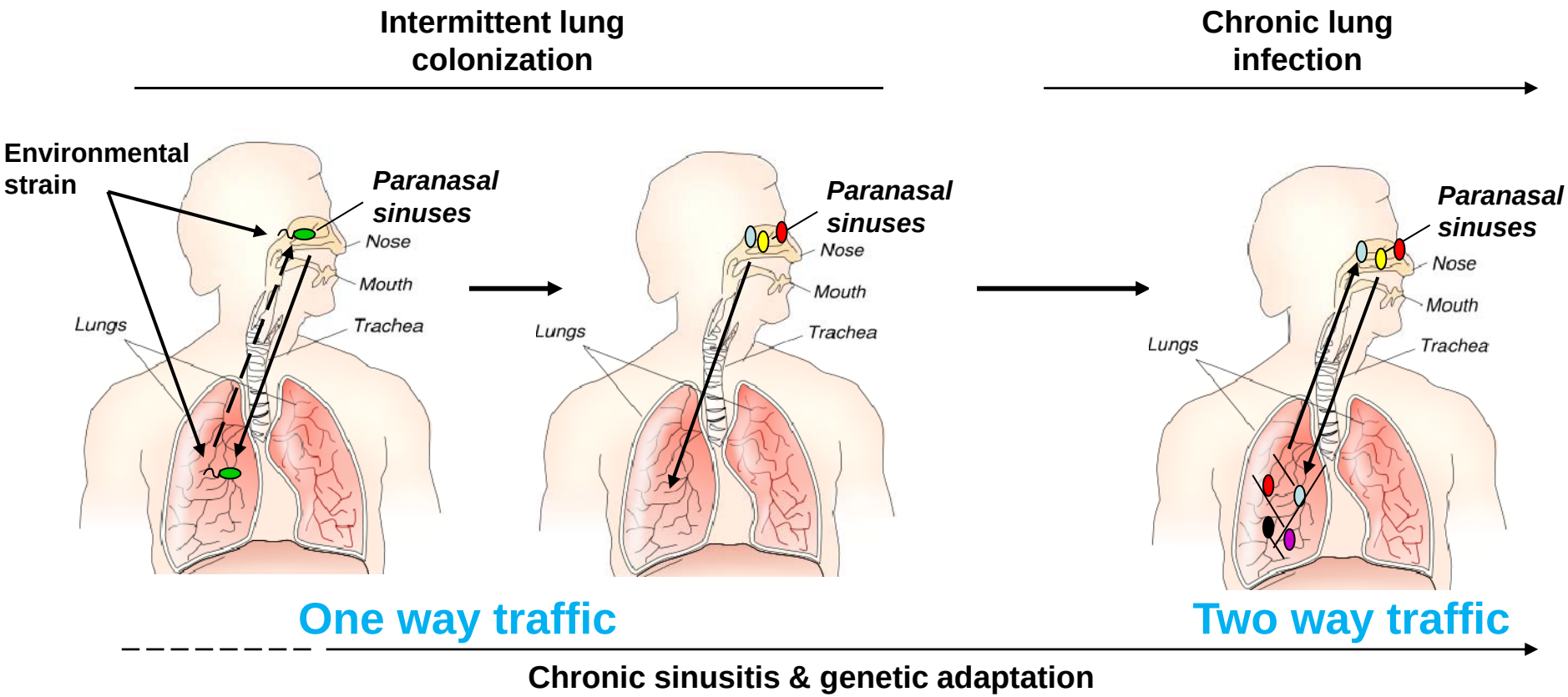


• CF patients typically develop persistent *Pseudomonas aeruginosa* lung infections that lead to reduced lung function.





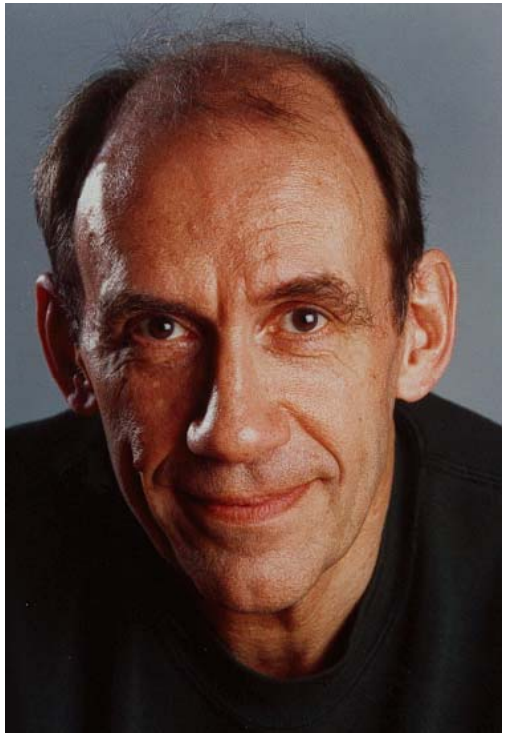
The Infection Process



: Diversification

’Experimental Evolutionary Biology’ at the Copenhagen Cystic Fibrosis Clinic

Niels Høiby founded in 1972 a unique collection of *Pseudomonas aeruginosa* bacteria isolated from CF patients with severe lung infections. This collection is still expanding.



Niels Høiby

Høiby’s strain collection is a gold mine of ’fossils’ for investigations of evolution in microbial populations

Competition experiments

which would never be permitted

Infection dynamics in chronically infected CF patients

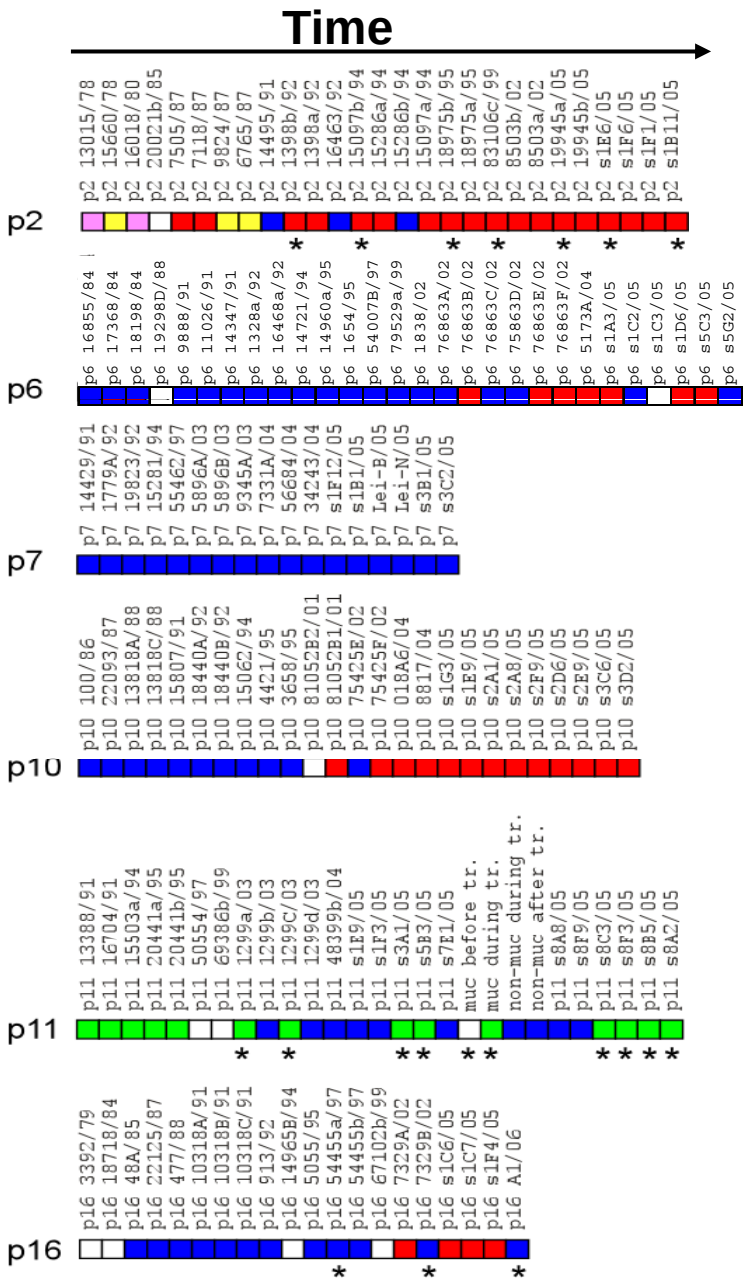
Genotype key:

Each Color indicates specific genotypes

No color indicates unique genotypes found only once

Phenotype key:

*** = mucoid**



Conclusions:

Two dominant clones: DK1‘red’ and DK2‘blue’

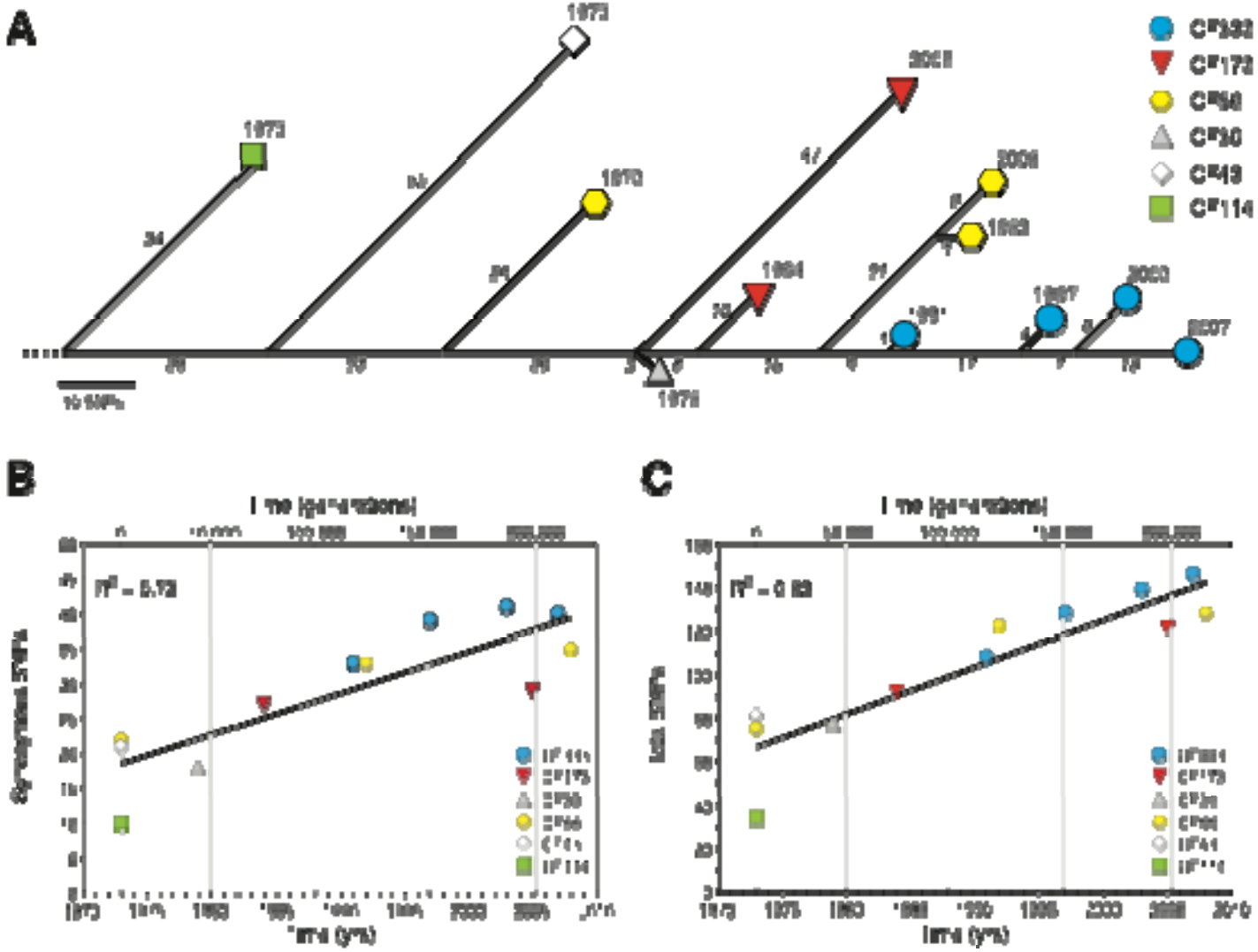
6/6 random patients have become infected with one or both

Most long-term chronic infected patients carry DK1 and/or DK2: We have identified the DK2 clone in 43 patients

Parallel Evolution

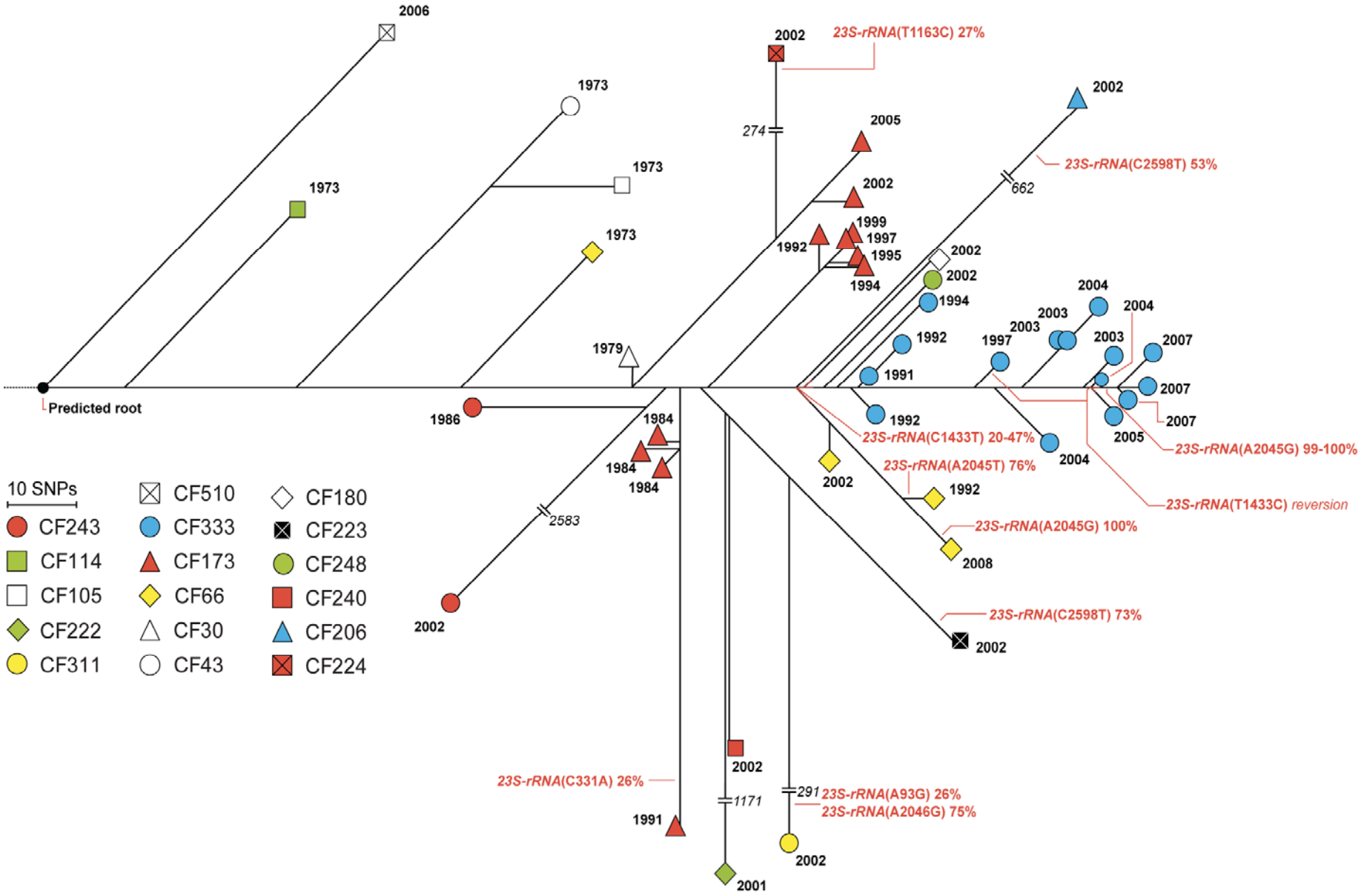
and phenotypic leaps

Accumulation of SNPs in ’blue’ lineage



Mutation rate: 7×10^{-11} / base pair / generation
Negative selection: dN/dS = 0.79

Parallel evolution of antibiotic resistance



Transcriptome Phenotypes
Experimental approach

in vitro growth in LB aerobically at 37ºC

cells harvested at mid-log phase

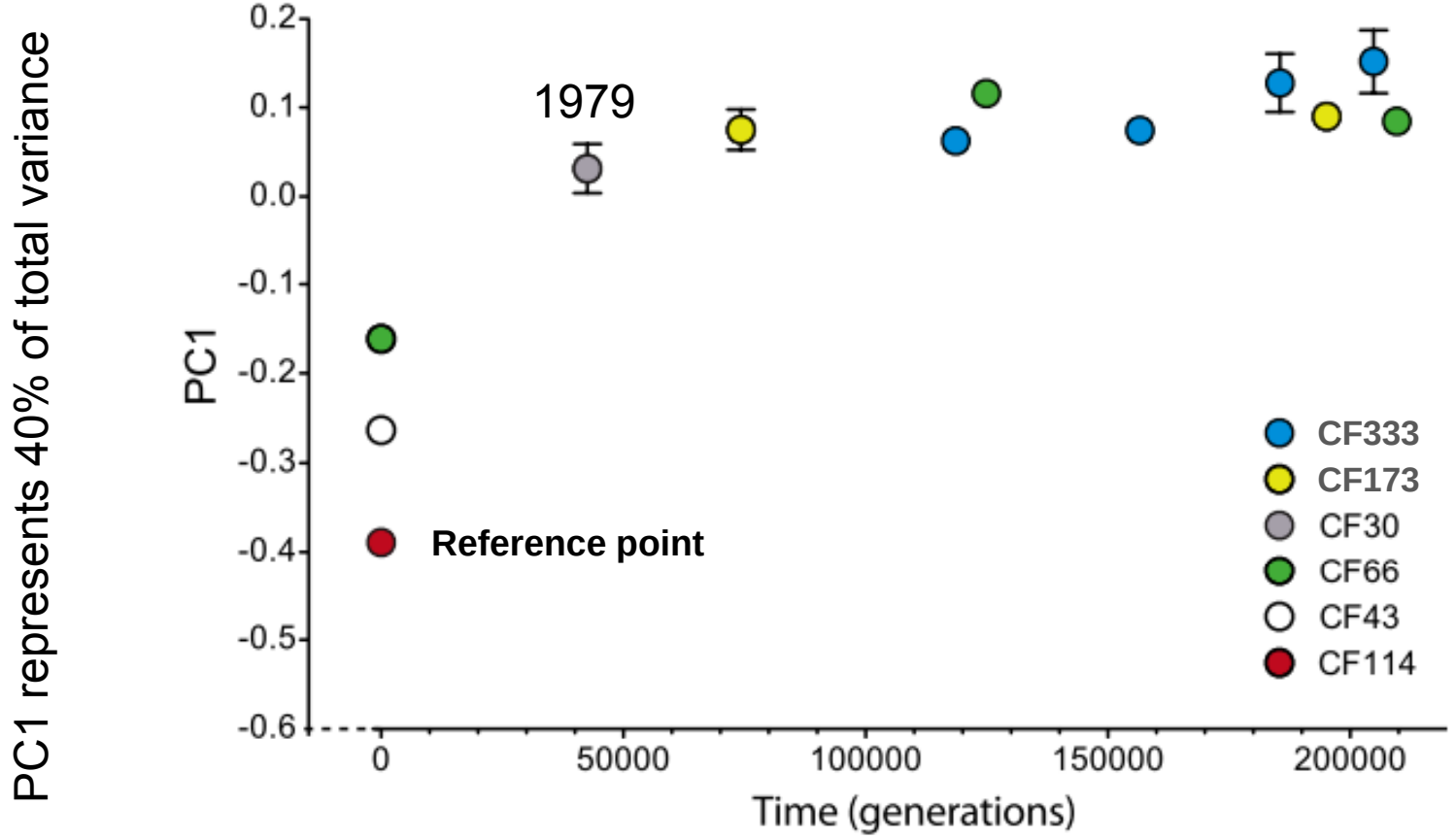
Affymetrix Genechip microarrays (PAO1)

data analysis

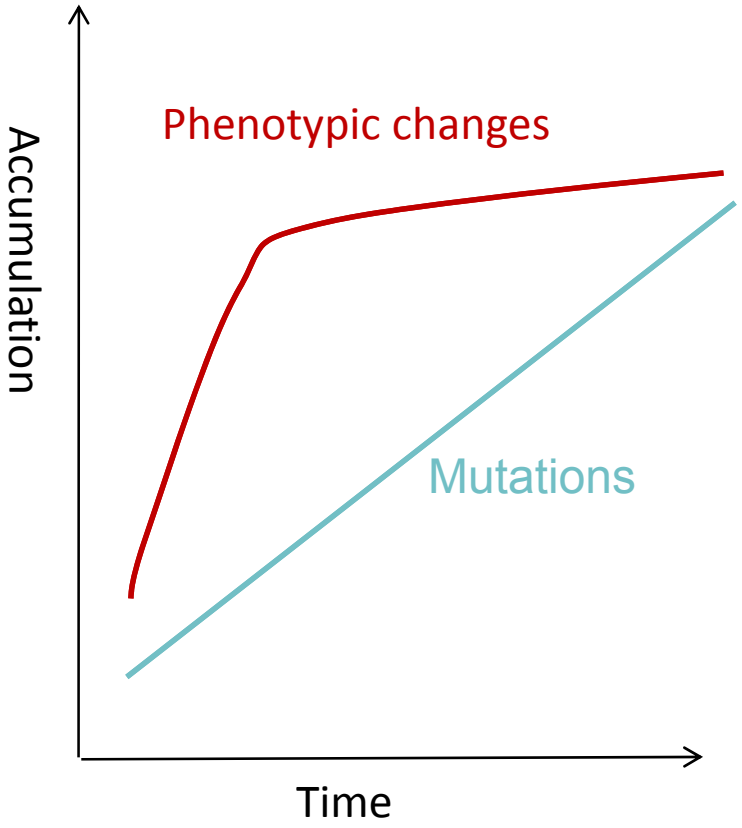
Results: An 'expression signature' for each strain analyzed

Changes in gene expression indicate mutations in control functions

Principle Component Analysis of Transcriptome data
Early phenotypic leap



No direct correlation between accumulation of mutations and accumulation of phenotypic changes



Could be explained by early pleiotropic mutations

Important mutations in early isolates

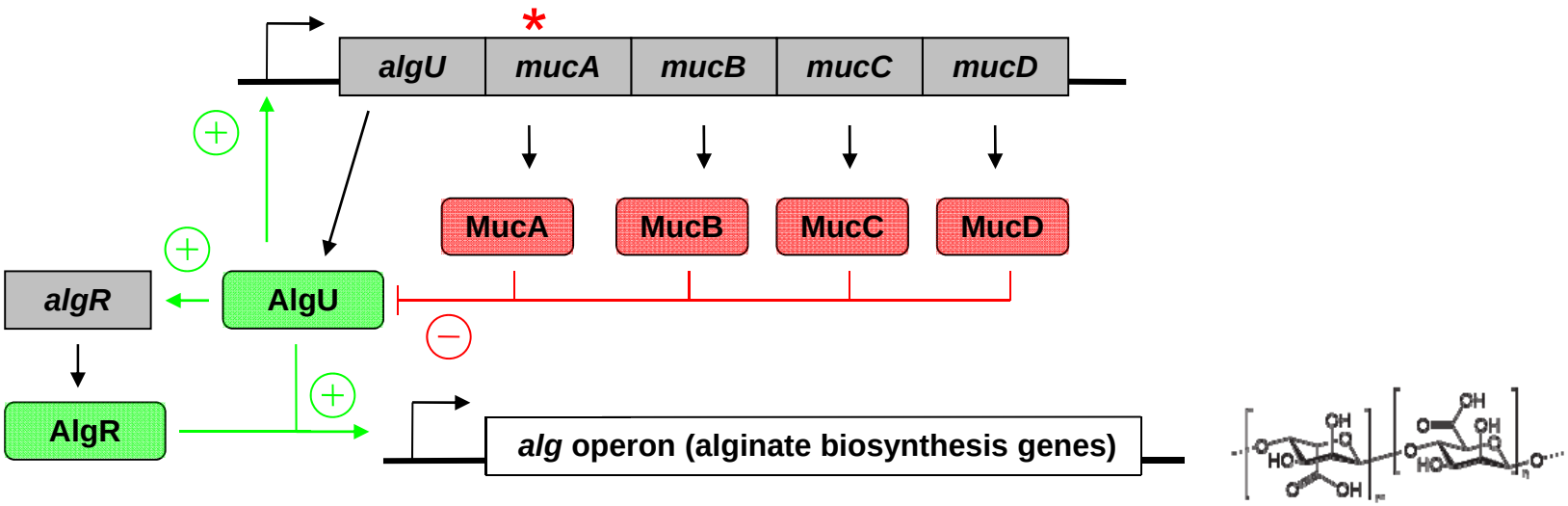
The isolate CF30-1979 which after 1979 was spread among multiple CF patients has mutations in the following regulatory genes:

	Locus tag	Name	
→	PA0763	*mucA*	430ΔG
	PA4462	*rpoN*	L419P
	PA1430	*lasR*	Δ*lasR*
→	PA0762	*algU(T)*	K55E
	PA4777	*pmrB*[3]	
	PA2426	*pvdS*[1]	

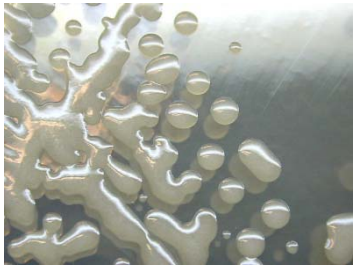
Locus tag	Name
PA2387	*fpvI*[1]
PA5200	*amgR*[2]
PA4102	
PA0120	
PA2020	

[1]Iron uptake via pyoverdine
[2]Stress response and antibiotic suscept
[3]Colistin resistance

The mucoid phenotype



**mucA* mutations are most frequent causes of mucoidy. Often frame-shift mutations.

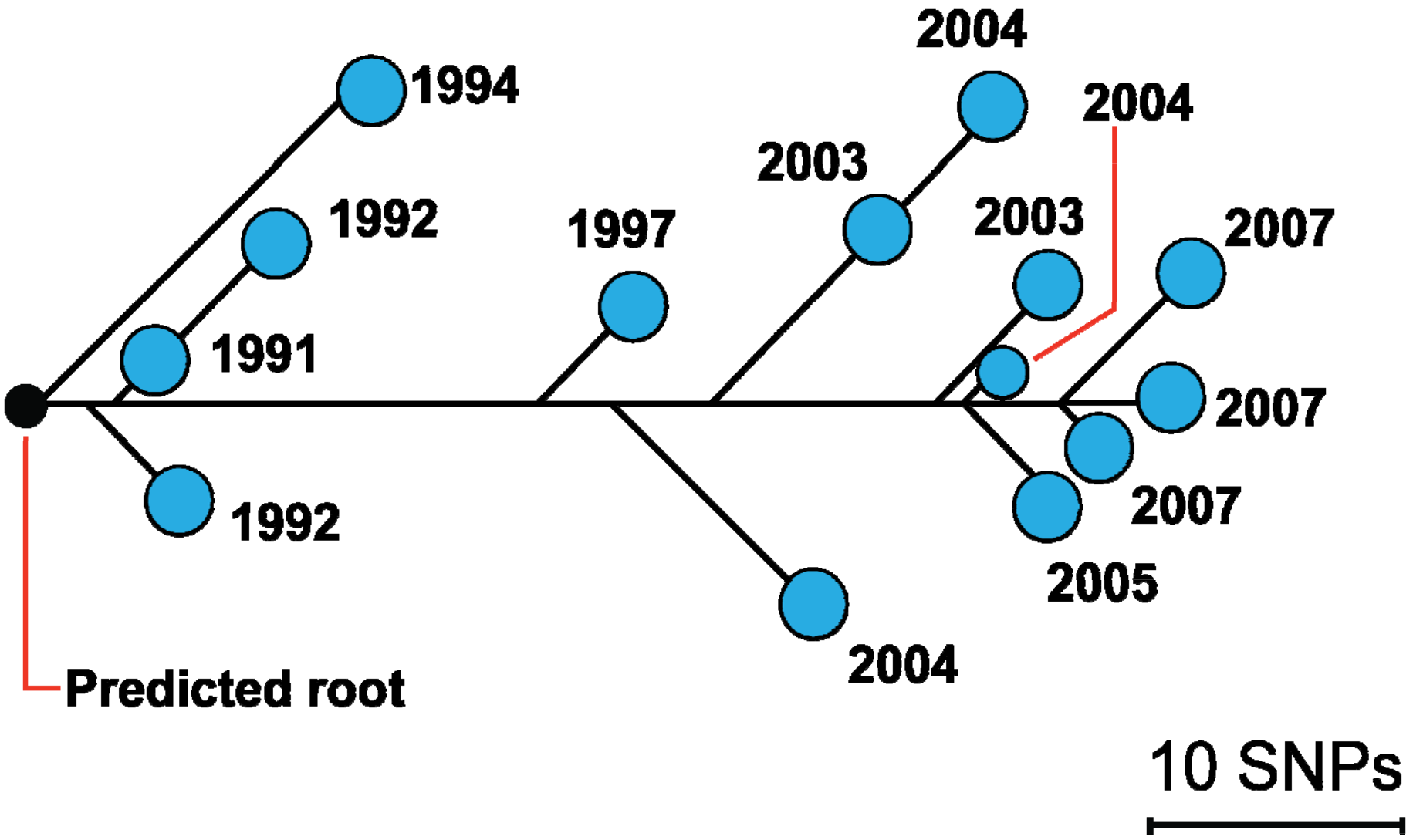


A Study of Evolution in a Natural Population
Preliminary Conclusions

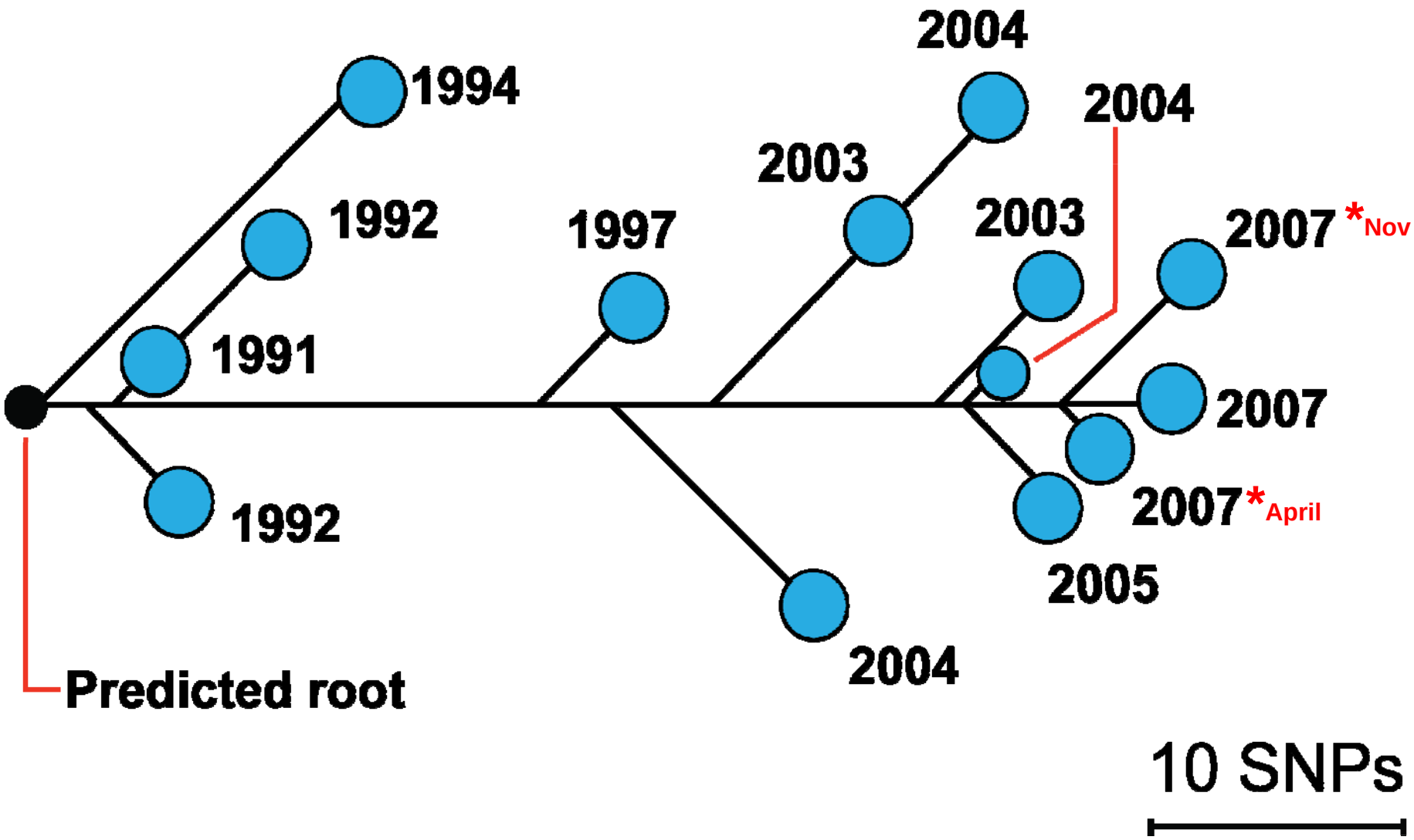
- **Successful transmissible and competitive lineage**
- **Constant low mutation rate over 200,000 generations**
- **Genetic drift more than adaptive evolution**
- **Early fast increase in fitness followed by constancy**
- **Pleiotropic mutations result in fitness peaking**
- **Low diversity despite structured environment**
- **Evidence for parallel evolution**

Evolution playing games with sigma factors

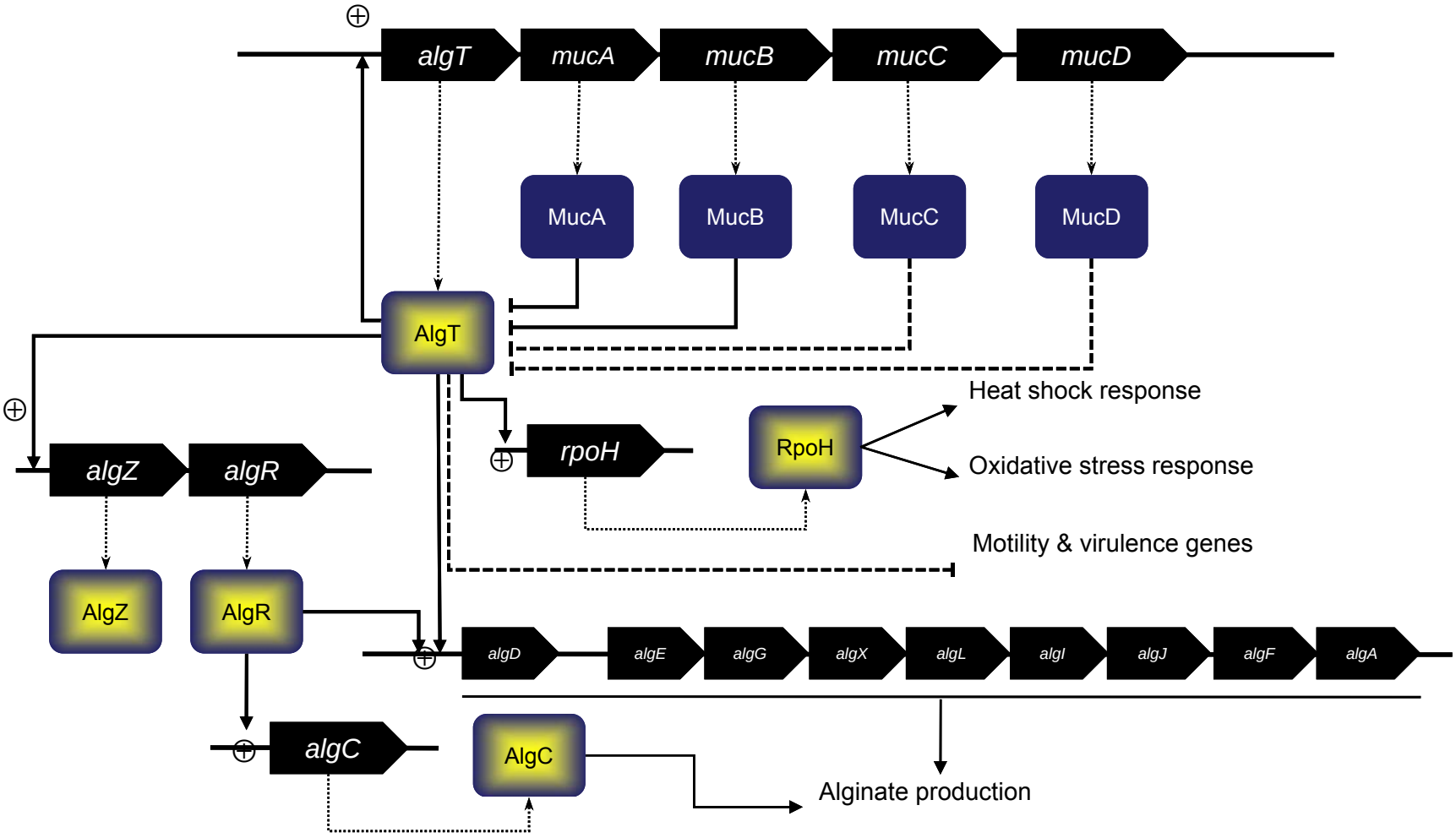
Evolutionary path in mono-clonally infected patient - all non-mucoid (*mucA, algU(T)*)



Late appearance of mucoid isolates



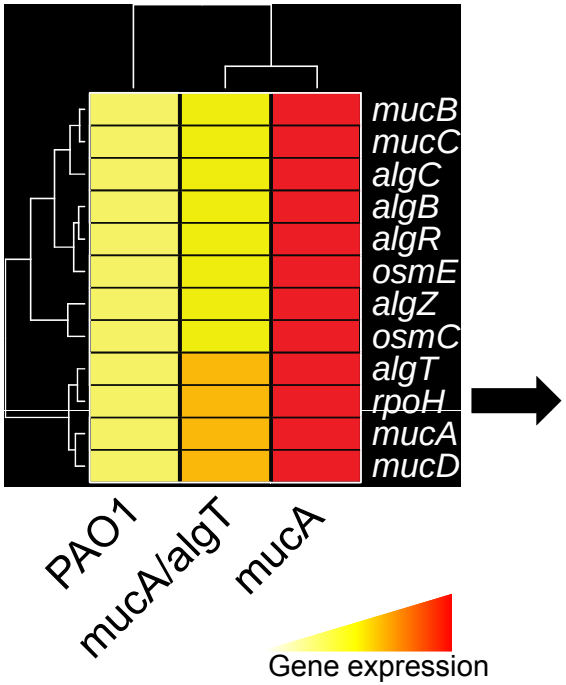
The *algT/mucA* regulon



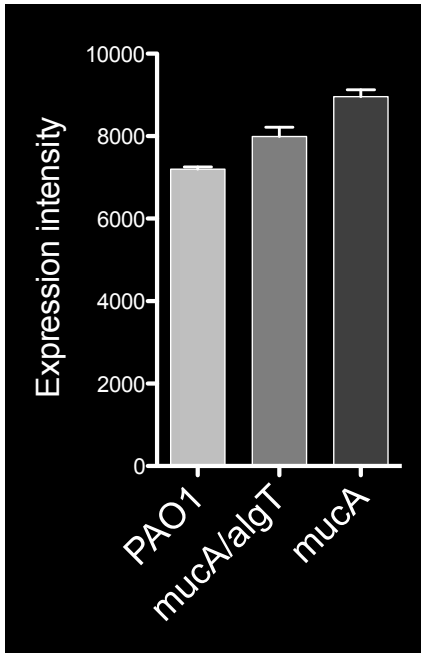
algT (G55A)

The G55A mutation leaves AlgT with partial activity…

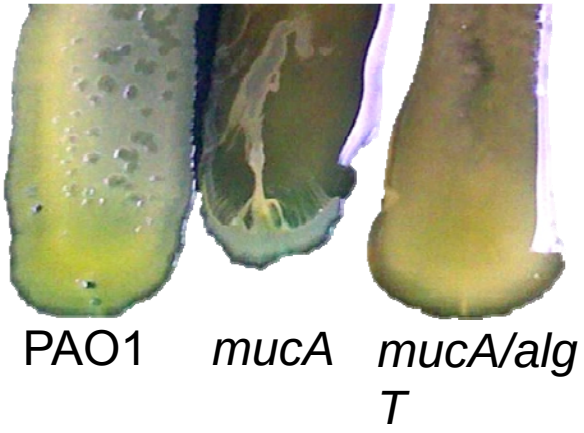
Expression of primary AlgT targets



Expression of *rpoH*

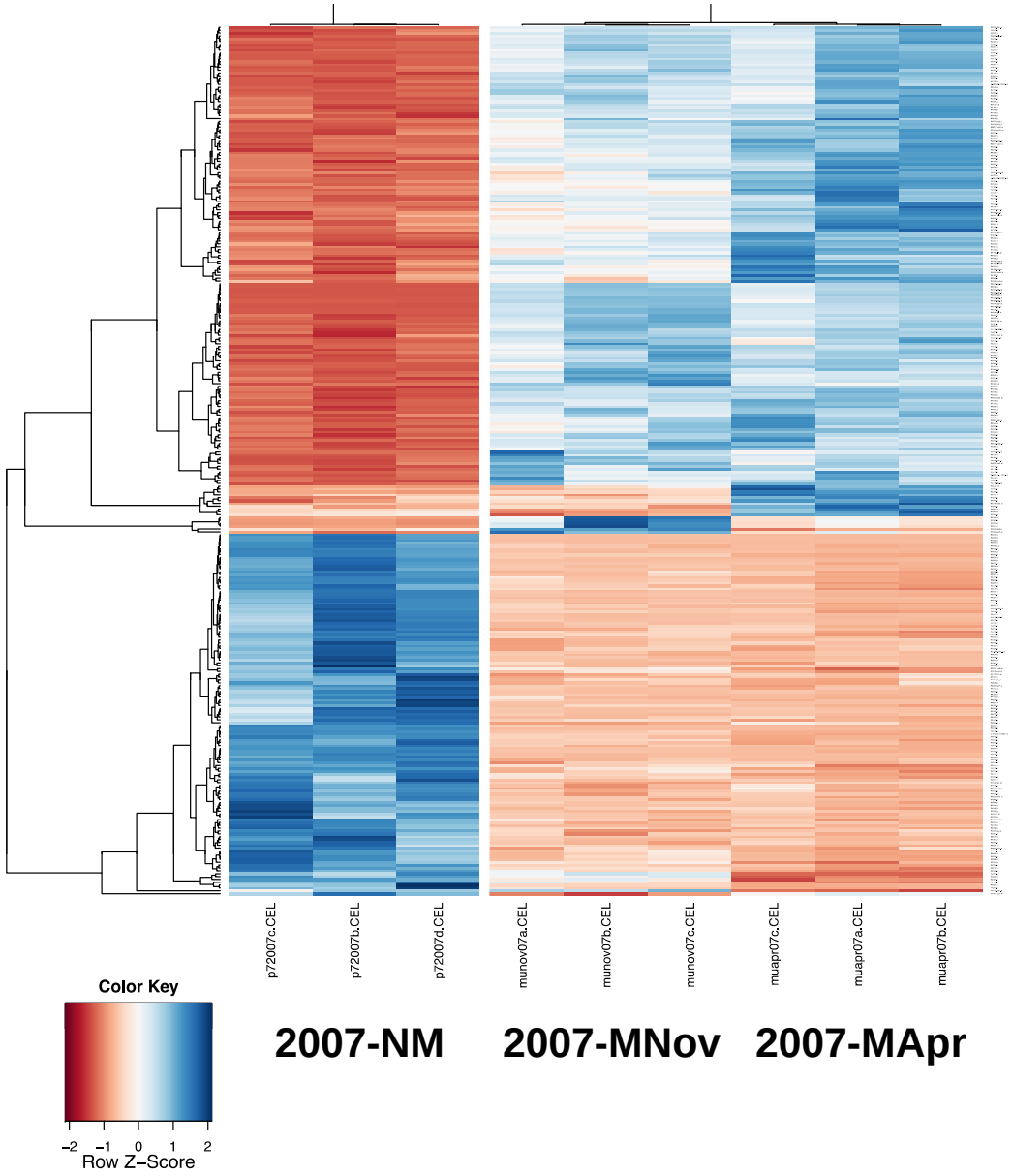


Alginate overproduction during osmotic stress?

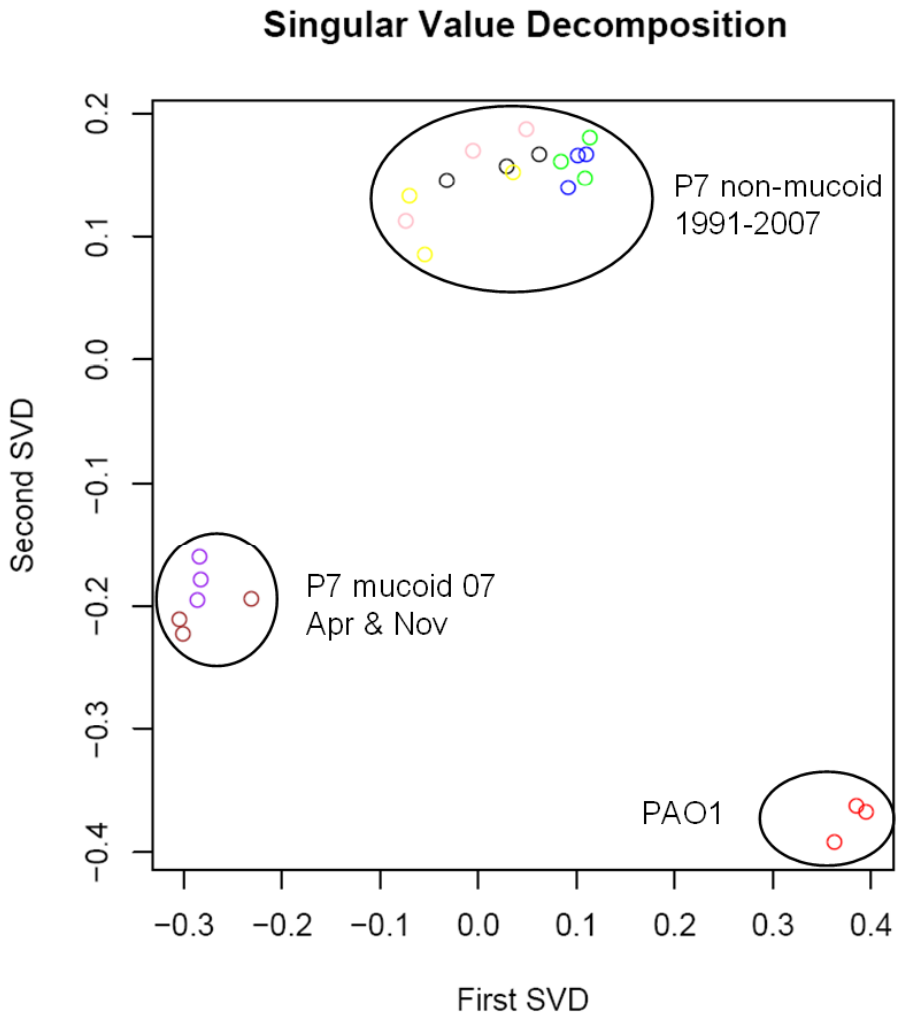


A *mucA/algT*^{G55A} mutant overproduces alginate when cultivated on LB supplemented with 8 % sucrose…

Heat map of gene expression in isogenic variants of DK2 from 2007 samples



Mutations causing mucoidy result in phenotypic leap



Mutations in mucoid isolates

	Gene ID	Gene name	Protein	mutation
P707Apr mucoid	PA2164		probable glycosyl hydrolase	G1735T;E579_
	PA4415	mraY	phospho-N-acetylmuramoyl-pentapeptide-transferase	G781A;A261T
	PA0576	rpoD ←	sigma factor RpoD	1517-1519deltaAAG
			No blast	Miscellaneous
P707Nov mucoid	PA4776	pmrA	two-component regulator system response regulator PmrA	T14C;L5P
	PA4418	ftsI	penicillin-binding protein 3	G524A;R175H
	PA2630		conserved hypothetical protein	C172A;R58S
	PA0436	?	probable transcriptional regulator	C426T;V142V, silent
	PA1802	clpX	ATP-dependent Clp protease ATP-binding subunit ClpX	C381T;T127T, silent
	PA0762	algU ←	Sigma factor AlgU	G55A; E19K

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- Genetic drift more than adaptive evolution
- Early fast increase in fitness followed by constancy
- Pleiotropic mutations result in fitness peaking
- Low diversity despite structured environment
- Evidence for parallel evolution
- Evidence for convergent evolution
- Phenotypics leaps at different time points

If endless stress is the challenge

there may be many ways to survive,
if evolution can play with 600 regulatory genes

Acknowledgements

- **Lei Yang**
- **Rasmus Marvig**
- **Søren Damkiær**
- **Lars Jelsbak**

- **Helle K. Johansen**
- **Niels Høiby**